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REDUCTION BY ROOTS[1]

OSWALD SCHREINER AND M. X. SULLIVAN

The roots of many plants, such as wheat, have an oxidizing power (1). This property is readily shown by certain chromogens, alphanaphthylamine, benzidine, phenolphthalin, aloin, guaiac, pyrogallol, etc. When chromogens like alphanaphthylamine and benzidine are used, the colors due to oxidation are shown on the root itself. The most marked oxidation is shown by a narrow but very distinct band of color just back of the root cap. Then comes a practically colorless zone and then a broad colored zone, the color becoming less intense toward the upper part of the root.

In regard to the practically colorless zone or the zone with little color just back of the region of intense coloration, it seemed as if the lack of color might be due to a reducing power, a reductase or possibly an antienzyme.

The possibility of an antienzyme is suggested by the work of BERTEL (2), who showed that with the exclusion of oxygen there accumulated in the cells of the lupine roots larger quantities of tyrosine than are usually found. The tyrosine which arises from the protein degradation in the early stages of the seedling's growth is normally oxidized by the enzyme tyrosinase to homogentisic acid and oxidation products of the latter. With the exclusion of oxygen, this oxidation is retarded and tyrosine accumulates.

Subsequently CZAPEK (3) showed that "a short time after the beginning of geotropic induction there appears a retardation of the normal destruction of tyrosine to be recognized by an accumulation of homogentisic acid." The cause of this retardation he attributed to the development of specific antioxidase which inhibits the normal activity of the oxidase of the root tip.

Reducing properties have been found in animal organs, in microorganisms, and in plant juices. The reducing power of the animal organism was shown first by EHRLICH (4), who used alizarin blue and the more easily reduced indophenol blue, both of which on reduction give colorless bodies. On injecting these colored substances he found that organs of the animal could reduce them to the leuco-bases. EHRLICH divided the organs of the body into three main groups: (1) those highly saturated with oxygen in which indophenol blue remains; (2) those in which indophenol blue, but not alizarin blue is reduced; (3) those of highest oxygen avidity, in which alizarin white is formed.

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In the first group are the gray matter of the nervous system, heart, part of the muscular system, and kidney capsule; in the second group, most of the muscular system, glands, and connective tissue; in the third group, the lungs, liver, fat, Harder's gland, and mucosa of the stomach.

Since EHRLICH'S experiments, the reducing power of animals, plants, and microorganisms has been extensively studied. The reagents used to demonstrate the reducing power are numerous. Among them are various colored substances which form leuco-bodies, such as lacmus, methylene blue, indigo carmine, indigo blue, gentian violet, methyl violet, rosaniline, etc.; nitrates, which are reduced to nitrites; sodium selenite and sodium tellurite, which are reduced to metallic selenium and tellurium. Methylene blue and lacmus are used most. The former is converted to a leuco-base by the addition of hydrogen, the latter apparently by the abstraction of oxygen.

Reduction has been studied more extensively in the case of microorganisms than in animals and plants. Whether the reduction produced by microorganisms is due to enzymes or not has not yet been settled. ROZSAHEGYI (**5**), BAGINSKY (**6**), MÜLLER (**7**), and WOLFF (**8**) believe that the reduction is caused by external metabolic products; while CAHEN (**9**), SPINA (**10**), SMITH (**11**), KLETT (**12**), MAASSEN (**13**), and CATHCART and HAHN (**14**) regard it as an attribute of the bacterial cell. They all agree, however, that the reduction is associated with the development of bacteria.

In regard to reduction in higher plants, it might be mentioned that BINZ and SCHULZ (**15**) found that plant protoplasm had the power of reducing arsenic acid (As_2O_5) to arsenious acid (As_2O_3), while plant protoplasm treated with boiling water lost this power to a great degree. REY-PAILHADE (16) extracted from yeast cells by means of alcohol a substance which would form H_2S from S. To this substance he gave the name philothion. He found it likewise in fresh animal tissues, in the tips of young shoots of asparagus (17), and in various grains and young seedlings (18). POZZI ESCOT (19) verified REY-PAILHADE'S work and succeeded in hydrogenating in addition metallic selenium and phosphorus. PALLADIN (20) found a reductase in wheat shoots and concluded that it played a part in respiration. DELEANO (21) found a reductase or hydrogenase in the albumen of *Ricinus communis*, but not in the roots or the plantules. The albumen triturated with sulphur gave hydrogen sulphide. LAURENT (22) showed that young seedlings reduce nitrates and that there can be extracted from plants an unorganized ferment which has the power to convert nitrates to nitrites. ABELOUS and ALOY (23) found a nitrate-reducing enzyme in potato tubers. KASTLE and ELVOVE (24) confirmed the presence of the nitrate-reducing enzyme in the potato and showed that it was present also in the fruit of the egg plant (*Solanum melongina*). Recently IRVING and HANKINSON (25) have found nitrate-reducing enzymes in certain water plants.

Since little has been done upon the reducing power of seedlings growing in soil or solutions, experiments were made to determine the power of the intact and growing roots, especially of wheat, to reduce substances, with the ultimate purpose of seeing if, like the oxidative power, the reducing power would be found to play a significant part in soil fertility.

Various dyes such as methylene blue, indigo carmine, Bismarck brown, gentian violet, etc., were first employed to see if the roots would decolorize the solution in which they were growing. The color of dilute solutions of the dyes was greatly lightened by the growing roots, especially if air was excluded. Since, however, more or less of the dye-stuff was deposited on the outer surface of the root and root hairs, such solutions were not considered satisfactory for showing reduction if this occurs.

STARCH IODIDE SOLUTION.—When wheat seedlings are placed in dilute solution of blue starch iodide with seeds and roots in the solution, the blue color is soon discharged. With the roots only in the starch iodide solutions, the color is discharged slowly. The addition of hydrochloric acid to the decolorized solution brings back the blue color. Reducing agents such as sodium thiosulphate, formaldehyde, hydrogen sulphide, etc., behave similarly.

Seedlings were kept in water solution of iodine of 0.012 per cent strength of iodine for two hours with: (*a*) seeds and roots in iodine solution; (*b*) roots only in iodine solution; and (*c*) control in which no plants had been. Solution (*a*) gave no color on addition of the starch; (*b*) gave a slight blue color; and (*c*) gave a deep blue.

On addition of a few drops of concentrated hydrochloric acid, all the solutions became deep blue and no difference could be seen in the depth of color. It would seem then that the plants hydrogenate the iodine, forming an iodide from which the acid liberates the iodine, or that the plants in some way render the iodide inactive toward the starch.

Since the chemistry of the starch iodine combination is not sufficiently known, and since the intensity of color produced by the reaction between starch paste and iodine is by no means quantitative, the effect the seedlings had on this reaction was taken merely as a sign of the possibility of a hydrogenase or a reductase in the seedlings, seed, or root.

SULPHUR.—The roots and the seed and roots of the intact seedling were put in contact with well washed, precipitated sulphur, neutral, made slightly acid with hydrochloric acid, or slightly alkaline with sodium hydroxide. In no case was hydrogen sulphide detected by means of lead acetate paper. Alcoholic extracts of the crushed seedlings after the method of REY-PAILHADE (16), and POZZI-ESCOT (19) were likewise found to have no hydrogenating action on precipitated sulphur.

HEFFTER (26) found that compounds containing the sulphydryl or SH group such as thiophenol, benzylmercaptan, ethylmercaptan, thioglycolic acid, and cystein reduce sulphur, and came to the conclusion that the reducing power of cells is due to the labile hydrogen of sulphydryl compounds which give up their hydrogen and become disulphides. This sulphydryl group is present in certain albumins, and is shown by a purple-red coloration with sodium nitroprusside and sodium or ammonium hydroxide. The crushed wheat roots or seedlings do not give this reaction. According to HEFFTER, non-reducing albumins can be changed to reducing albumins by treatment with a 10 per cent sodium sulphite solution for 12–24 hours and will then give the nitroprusside reaction.

Wheat seedlings of different stages of development from three to ten days old were rapidly crushed, covered with a 10 per cent solution of sodium sulphite, and kept at 40° C., with repeated shaking. The solutions with suspended matter were tested after 2, 6, and 24 hours, and at no time gave the nitroprusside reaction for the sulphydryl group nor did they reduce sulphur. In 24 hours the seedling pulp was tested for the sulphydryl group with negative results, and the pulp formed no hydrogen sulphide from freshly precipitated sulphur.

NITRATES.—To test the power of wheat seedlings to reduce nitrates to nitrites, the wheat grains were treated with a 0.1 per cent solution of mercuric chloride for 30 minutes, and the seeds, well washed with sterile water, were placed in sterile tubes containing a little water. In the tubes tightly closed with cotton the wheat germinated and grew well. There were added to each tube 10 cc. of a 1 per cent solution of potassium nitrate, and after 24 hours the solution was tested for nitrites by means of Griess reagents (**27**), sulphanilic acid and naphthylamine acetate. The ungerminated seeds, whether sterilized or not, did not give the nitrite test under these conditions. The solutions containing the seedlings, on the other hand, gave good tests for nitrites. The ability of sterile seeds germinated in tubes to form nitrites from nitrates was tested several times, both in air and in vacuum. Sometimes a fair amount of nitrite was formed, in one case as high as 6 parts per million, sometimes the amount was very little. It should be borne in mind, however, that nitrites are absorbed by plants. The formation of nitrites was greater in vacuum. It would seem that a nitrate-reducing power appears in the early stages of the wheat seedling, but is not in the seed at rest. The formation of nitrites is small, and no culture tests were made to show that microorganisms were entirely excluded in the experiment. It may be said, however, that enzymes capable of reducing nitrates have been reported in plant tissue by LAURENT (**22**), ABELOUS and ALOY (**23**), KASTLE and ELVOVE (**24**), and IRVING and HANKINSON (**25**). LAURENT claims that young seedlings of maize, white lupine, and peas have a reducing action on nitrates, while IRVING and HANKINSON have found that a number of plants reduce nitrates more or less.

SODIUM SELENITE.—Sodium selenite was next employed as a test solution. Solutions of sodium selenite of 0.25 per cent were first used. The solutions reacted alkaline to litmus and phenolphthalein, and were toxic to the young seedling. When neutralized by hydrochloric acid, however, the toxicity of the selenite was greatly lessened. When seedlings were grown in the sodium selenite solution made neutral or slightly acid to phenolphthalein and of a strength of 0.125 to 0.25 per cent, the parenchyma cells of the end of the root cap were colored an intense pink in a few hours, varying with the seedlings, by the deposit of selenium. This deposit was more marked in slightly acid solutions. The points of emergence of the secondary roots were likewise colored. Later the whole root became colored.

SODIUM TELLURITE.—When sodium tellurite is used as a test for reduction, the roots are colored a blue-black by the deposit of metallic tellurium, otherwise its behavior is like that of sodium selenite.

These experiments show conclusively that the intact roots possess a reducing power. This reducing power is stronger in the young seedling, being much stronger in the seedling 4 days old than in the seedling 12 days old. As judged by the quickness with which the deposit of selenium is made on the roots and the extent and intensity of the deposit, the reducing power increases from time of germination to the sixth or eighth day and then decreases. It is still present in seedlings 13 days old, the oldest seedlings examined. The oxidizing power of the wheat seedlings, on the other hand, as judged by the oxidation of aloin, is less in the young seedling and increases with age, being considerably greater in the twelve-day seedling than in the six-day seedling, and still greater than in the case of the four-day seedling.

Dying tissue tends to have a reducing action. The reducing action of the wheat roots on sodium selenite, however, is not a death phenomenon, since (1) roots killed by being dipped in boiling water have no reducing action on the selenite; (2) the roots in toxic non-neutralized sodium selenite do not reduce; (3) intact roots boiled in solution of sodium selenite have no deposit of selenium upon them; (4) the root ends being cut off and the injured root placed in the selenite solution, the deposit of selenium is not on the cut end but at the point of emergence of the secondary roots.

The reducing power of the wheat roots on sodium selenite is checked by acids and alkalies and by toxic organic matter. It is stimulated by faintly acid reaction and by light. The observable action of salts on the reducing action is variable on account of lack of precision of method, though as a rule sodium nitrate solution equivalent to 50 p.p.m. NH_3 gives the quickest and heaviest deposit at the root tip, while potassium salts give the heaviest deposit on the rest of the root. Potassium salts containing 50 p.p.m. K_2O appeared to retard reduction slightly at the root tip. Potassium iodide containing 14.5 p.p.m. K_2O, however, stimulated reduction.

Incidentally it is of interest here to state that potassium retards greatly the oxidation power as indicated by aloin, and that a slight acid reaction stimulates reduction while inhibiting oxidation, thus seeming to show that the reducing and oxidizing powers are not necessarily concomitant.

Whether or not the reducing power of the wheat roots on sodium selenite is due to enzyme activity, it is hard to say positively. In regard to the enzyme nature of the reducing agent in animal cells, HEFFTER (**26**) concluded that there were two groups of reduction processes. One group was hardly influenced by hydrochloric acid or by heating. This group embraced the formation of hydrogen sulphide from sulphur, the reduction of arsenic, tellurium, and selenium compounds, the reduction of cacodylic acid and of different dye-stuffs. These reductions he believes are caused by the labile hydrogen of the sulphydryl group of certain albuminous bodies. The other group of reduction processes, such as the change of nitrates to nitrites and of nitrobenzol to amino combinations, were restrained by heat. This group of reactions he thought at first might be enzymotic, but later he came to the conclusion (**26**) that they likewise were non-enzymotic.

Whether enzymotic or not, the reducing action on sodium selenite is most marked intracellularly in the parenchyma cells of the root tip. The primary localization of the deposited selenium in the root tip speaks neither for nor against the enzyme nature of the reducing power.

Reduction of sodium selenite may be brought about by purely chemical means. Thus GRÜSS (**28**) found that lactic acid could precipitate selenium from sodium selenite. Lactic acid we have found to have no reducing action on sodium selenite of a strength of 0.25 per cent, but has some reducing action on a 4 per cent solution of sodium selenite, especially on warming. Citric, tartaric, malic, and oxalic acids do not reduce sodium selenite in the cold, but do reduce strong solutions of the selenite on boiling with a slight excess of the acid. Hydrochloric acid has no reducing action on the selenite solution. Citric, tartaric, malic, and oxalic acids have a reducing action likewise on ferric salts, converting them to the ferrous form. Unsaturated fatty acids such as oleic and elaidic also have the power to reduce sodium selenite and tellurite to metallic selenium and tellurium on warming the mixtures on the water bath. Dextrose likewise reduces the selenite and tellurite on warming. A strong solution of sodium selenite mixed with a solution of invert sugars made by warming cane sugar with dilute hydrochloric acid was completely reduced in the slightly acid mixture on standing 48–72 hours at the room temperature of about 30° C.

JONES (**29**) injected selenate into the blood of animals. The selenate was reduced to selenite, a small part of which was excreted in the urine. The remainder was carried to the spleen and liver, where it was reduced by dextrose to selenium. When the dextrose was exhausted, fat was called upon. JONES suggests that dextrose is possibly the means by which all reduction processes in the body are brought about. He found that arabinose, glucose, and sugars yielding glucose would reduce sodium selenite on heating, while levulose could reduce it at 30° C. on long standing.

As regards the reduction of sodium selenite and tellurite by wheat roots, it seems probable, since no reducing enzyme could be extracted from the crushed plant, although the juice did show reduction of selenite upon heating, that the reduction is due to the metabolic activities of the roots, to some unstable non-enzymotic bodies comparable to the oxyorganic acids, which have a slight reducing power, or to complex, unsaturated compounds comparable to dextrose and levulose or the unsaturated fatty acids.

In regard to the significance of the reducing power, it may be said that reduction in organisms runs in general concurrently with oxidation, and is probably just as important an index of life activity. A better medium than sodium selenite is required, however, for a detailed study of this reducing power. Such a medium would be a chromogen which changed color under the reducing action, and thus would present a possibility of estimating the change colorimetrically and quantitatively. The fact stands, however, that the growing root possesses reducing powers.

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